

Claims

1. A method of determining the frequency of an allele in a population of nucleic acid molecules, said method comprising:
- 5 pooling the nucleic acid molecules of said population, performing primer extension reactions using a primer which binds at a predetermined site located in said nucleic acid molecules, and obtaining a pattern of
- 10 nucleotide incorporation.
2. The method according to claim 1 wherein the primer extension reaction is performed by sequentially adding nucleotides to the reaction mixture and determining the
- 15 incorporation or non-incorporation of each nucleotide.
3. The method according to claim 2 wherein the amount of nucleotide incorporated is determined quantitatively.
- 20 4. The method according to claim 3 wherein the nucleotide is detected by detecting the release of pyrophosphate.
5. The method according to claim 4 wherein ELISA
- 25 detection enzymes are used to detect the release of pyrophosphate.
6. The method according to claim 5 wherein a nucleotide-degrading enzyme is included during the
- 30 primer extension reaction.
7. The method according to claim 1 wherein the nucleic acid molecules are immobilized on a solid support.
- 35 8. The method according to claim 1 wherein the amount or concentration of the nucleic acid in each sample of the population which is pooled, is determined prior to pooling.

9. The method according to claim 8 wherein the concentration of the nucleic acid in the sample is determined by a primer-extension reaction.

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10. The method according to claim 9 wherein the volume of each nucleic acid in each sample to be pooled is adjusted in view of the amount or concentration of nucleic acid present such that the pooled sample
10 contains substantially the same amount or concentration of each nucleic acid molecule in the population.

11. The method according to claim 10 wherein in order to perform said calibration a particular polymorphism is
15 selected as a reference (or marker) and said primer extension reaction is specific for said reference polymorphism.

12. The method according to claim 11 wherein said
20 polymorphism is chosen such that it gives no background signals in a primer-extension reaction and that the signals are even.

13. The method according to claim 11 wherein said
25 polymorphism is not present in a homopolymeric sequence and will not preferentially amplified in any PCR-type reactions.

14. The method according to claim 11 wherein a
30 reference sample is selected as the main reference from one of the homozygotes of one of the alleles of said polymorphism (Ref 1) and another reference (Ref 2) is selected from the other homozygote, and the reference samples are pooled and primer extension reactions are
35 performed as described in claim 1, and the pattern of nucleotide incorporation determined to determine the relative concentration of each reference sample.

15. The method according to claim 14 wherein the sample nucleic acid molecule to be tested are pooled individually with the reference samples.

5 16. A kit for carrying out the method according to claim 1 comprising optionally primer(s) for *in vitro* amplification; a primer for the primer extension reaction; nucleotides for amplification and/or for the primer extension reaction; a polymerase enzyme for the
10 amplification and/or primer extension reaction; and means for detecting primer extension.

17. A method of determining the amount of an allele in a sample of nucleic acid molecules, said method
15 comprising:

performing primer extension reactions on said nucleic acid molecules, using a primer which binds at a predetermined site located in at least one said molecule, and determining which and/or how many
20 nucleotides are incorporated in said reaction, and analysing said nucleotide incorporation information thus obtained in order to determine the amount of occurrence of said allele in said sample.

25 18. The method according to claim 17 wherein the primer extension reaction is performed by sequentially adding nucleotides to the reaction mixture and determining the incorporation or non-incorporation of each nucleotide.

30 19. The method according to claim 18 wherein the amount of nucleotide incorporated is determined quantitatively.

20. The method according to claim 19 wherein the nucleotide is detected by detecting the release of
35 pyrophosphate.

21. The method according to claim 20 wherein ELIDA detection enzymes are used to detect the release of pyrophosphate.

22. The method according to claim 21 wherein a nucleotide-degrading enzyme is included during the primer extension reaction.

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23. The method according to claim 22 wherein the nucleic acid molecules are immobilized on a solid support.

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